Hormonal spawning induction and larval rearing of meagre, *Argyrosomus regius* (Pisces: Sciaenidae)

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The aim of the present study was to evaluate the culture potential of meagre, *Argyrosomus regius* (Asso, 1801). Trials were conducted in two different facilities in Spain, the LIMIA research centre (Mallorca) and the IFAPA research centre "El Toruño" (El Puerto de Santa Maria). In May 2006, males with free milt and females with vitellogenic oocytes bigger than 500 µm were injected with salmon gonadotropin releasing hormone analogues (sGnRHa). Spawning occurred approximately 38 hours after induction. Fecundity was high with 1.207.000 eggs collected from a 13 kg female at LIMIA in a single spawn. The larval development, growth and morphological changes were described from 0 to 30 days post hatching (DPH). The mean length of the newly hatched larvae ranged from 2.20 ± 0.02 mm (LIMIA) to 3.19 ±0.09 mm (IFAPA). Feeding began on 3 DPH, initial swim bladder inflation was observed on 5 DPH, and metamorphosis was completed on 30 DPH. Growth was very fast and the post-larvae reached 15.11 ± 3.49 mm (LIMIA) and 11.66 ± 0.96 mm (IFAPA) in 30 days Cannibalism was observed from 15 DPH onwards. These preliminary results indicate the meagre as a priority species for the diversification in aquaculture.

Keywords: Argyrosomus regius, *Aquaculture, spawning induction, Reproduction, Hormones, Larvae.*

INDUCCIÓ HORMONAL A LA POSTA I CULTIU LARVARI DE LA CORBINA, *Argyrosomus regius* (Pisces: SCIAENIDAE). L'objectiu de l'estudi va ser avaluar el potencial del cultiu de la corbina, *Argyrosomus regius* (Asso, 1801). Les experiències es van realitzar en dues diferents instal·lacions a Espanya. El centre d'investigacions LIMIA (Mallorca) i el centre d'investigacions IFAPA "El Toruño" (El Port de Santa Maria). Al maig del 2006, mascles fluents i femelles amb ovòcits vitel·logènics majors de 500 micres van ser injectats amb anàlegs de l'hormona alliberadora de gonadotropina del salmó (sGnRHa). La posta es va obtenir aproximadament 38 hores després de la inducció. La fecunditat va ser alta, obtenint-ne 1.207.000 ous recollits d'una sola femella de 13 kg en una única posta. El desenvolupament larvari, creixement i canvis morfològics es van descriure des del dia 0 al 30 després de l'eclosió (DPH). La longitud mitjana de les larves recent eclosionades va variar entre 2.20 ± 0.02 mm (LIMIA) i 3.19 ± 0.09 mm (IFAPA). L'alimentació va començar el 3 DPH, la inflació inicial de la bufeta natatòria es va observar el 5 DPH, i la metamorfosi es va completar el 30 DPH. El creixement va ser molt ràpid aconseguint les post larves 15.11 ± 3.49 mm (LIMIA) i 11.66 ± 0.96 mm (IFAPA) en 30 dies. A partir del 15 DPH es va observar canibalisme. Aquestes dades, assenyalen a la corbina com una espècie prioritària en la diversificació de l'aqüicultura.

Paraules clau: *Argyrosomus regius, Aqüicultura, Inducció a la posta reproducció, hormones, larves.*

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Introduction

The meagre, *Argyrosomus regius* (Asso, 1801), is a member of the family Sciaenidae, commonly refered to as croakers and drums. This family includes about 70 genera and 270 marine, brackish and fresh water species distributed all over the world (Nelson, 2006). Meagre is widely distributed along the Atlantic coast (northward to southern Norway and southward to the Congo) and in the entire Mediterranean Sea (Chao, 1986). It is a coastal species that can be found both close to the bottom of the continental shelf and near the surface within a depth range of 15 to 200 m. Juveniles and subadults are common in estuaries and shallow coastal areas (Quero, 1985, Chao, 1986, Quero and Vayne, 1987; Griffiths and Heemstra, 1995). Several authors have suggested that meagre spawn in estuaries, where they often form spawning aggregations (Quero and Vayne, 1987; Quero, 1989; Lagardêre and Mariani, 2006; González-Quirós *et al.*, 2011). Meagre has a high commercial value and is also targeted by sport fishermen (Chao, 1986).

Sciaenids are generally considered good aquaculture species because they are widely distributed, euryhaline, highly fecund, fast growing and have good food conversion ratios (Silberschneider and Gray, 2008). There has been increasing interest in studying meagre not only as a possible candidate for diversifying commercial aquaculture but also for restocking the depleted natural fishery. The first studies were carried out in France and Italy, and studies began in Spain in 1996 (Calderón *et al.*, 1997). Pastor *et al.* (2002) obtained excellent results growing-on wild 111.8± 25.8 g meagre juveniles in sea cages, which reached 1850±244.9 g in eight months when fed on a diet of fish.

There has only been limited production of meagre fry, with a single private hatchery operating in France and four experimental public hatcheries operating in Spain since 2006 (Cárdenas *et al.*, 2008). The optimal rearing protocol has not yet been determined for this species (Angelini *et al.*, 2002; Quèmèner, 2002; Grau *et al.*, 2007; Duncan, *et al.*, 2008), however, there are many studies on the reproduction and rearing of other similar sciaenid species, such as mulloway *Argyrosomus japonicus* (Temminck and Schlegel, 1843), shi drum *Umbrina cirrosa* (L.) (Mylonas *et al.*, 2000), and red drum *Sciaenops ocellatus* (L.) etc. All of these species exhibit some form of reproductive dysfunction and can only be induced to spawn with hormonal treatments (Thomas and Boyd, 1988; Zohar and Mylonas, 2001).

Controlling the reproductive process is one of the bottlenecks in the development of commercial aquaculture (Zohar and Mylonas, 2001). In Spain, attempts to evaluate the possibilities of culturing

meagre started in the year 2000 with the capture of wild juveniles in salt marsh ponds of the Guadalquivir River. Here we describe our experience with this wild stock at two research centres using different protocols and conditions. We describe how we controlled the reproduction of the wild caught meagre adults and then reared the larvae. It is crucial to understand these processes in order to improve our knowledge of the biology of this species and determine its potential as a commercial species within the aquaculture industry.

Materials and methods

Capture of breeders for the two research centres

In the autumn of 2000, 94 wild juvenile meagre were caught in the Guadalquivir river estuary (western Andalusia, south coast of Spain). They were held for quarantine and acclimation for a period of six months in tanks supplied with continuous flow-through sea water. During this time they were fed on squid, fresh or frozen fish (*Sardina pilchardus* and *Trachurus trachurus*) and crabs. In April of 2001 the stock was divided into two groups: one with the 50 smallest individuals (111.8±25.8 g mean weight), which was transported in a van equipped with a 0.6 m^3 tank-supported with oxygen to the "Laboratori d'Investigacions Marines i Aqüicultura" (LIMIA), Port d'Andratx, (Mallorca, Spain) and stocked in sea cages; and one group with the remaining 44 specimens (339 ±58.7 g mean weight), which was held in a tank at the "Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica" (IFAPA), El Toruño, El Puerto de Santa María (Cádiz, Spain) (Table 1).

LIMIA (Balearic Islands)

Rearing of breeders

In Port Andratx fish were kept for five years in 700 m^3 sea cages where they were exposed to a natural thermal and photoperiod regime and fed on fresh or frozen fish (*Sardina pilchardus, Spicara smaris* and *Trachurus trachurus*). Food rations were given four days per week to apparent satiation.

In February 2003, six individuals (3 kg mean weight) were sacrificed for the histological examination of gonads in order to determine if the fish were maturing in cage captivity conditions. The gonad samples were fixed in 10% buffered formalin, embedded in paraplast, sectioned at 3-4 µm, and stained with haematoxililin-eosin for routine light microscopic examination. In December 2005, eleven individuals were selected as breeders, sampled (length and weight) and tagged with AVID microchips. This required capturing the fish with dip nets and transporting them individually in a 0.2 m^3 tank to onshore facilities where they were immersed in an anaesthetic bath of 0.07 g L^{-1} MS222 before processing. All individuals were returned to the sea cages following this procedure after they had recovered from the anaesthetic.

Hormonal induction

The broodstock were sexed in May 2006, which involved examining males for free milt and performing an ovarian biopsy on females with a 2.67 mm diameter plastic nasogastric Levin catheter. The fish were then induced to spawn using injections of Ovaprim (Syndel Laboratory Vancouver, Canada), a commercial product whose active ingredients are the analogue of salmon gonadotropin-releasing hormone [D-Arg^6, Pro^9, Nehylamide]–s GnRHa and

a dopamine inhibitor. It was administered intraperitoneally to the abdomen via the rear pelvic fin. One female and 2 male were injected with 10 µg kg^{-1} and 5 µg kg^{-1} of sGnRHa respectively. Both the female and male fish were re-injected after seven and fourteen days, always using the same doses of sGnRHa. During the induction period fish were held in a 10 m^3 indoor rectangular tank, separated of the rest of the broodstock, with continuously flowing sea water (3 $m^3 h^{-1}$) at salinity 37 g L^{-1}, ambient temperature (18.5° C) and fitted with an overflow collector for floating eggs which consisted of 500 µm mesh bags placed below the outflow from the spawning tank. Eggs were collected 6 hours after the spawning to ensure that all the spawn was in the eggs collector.

Larval rearing

Estimations of the number of floating eggs and hatch rates were made volumetrically by taking five 10 mL aliquots of well mixed eggs or larvae from 10 L glass containers and observing

HOLDING STRUCTURES AND ENVIRONMENT CONDITIONS		
Variable	**IFAPA**	**LIMIA**
Maintenance structures	Concrete Tanks	Cages
Number of structures	1	1
Volume (m^3)	250	700
Water depth (m)	3.5	5.0
Min.- max. temp. (°C)	9 - 28	13-28
Salinity (g l^{-1})	18 - 40	37
BREEDERS		
Variable	**IFAPA**	**LIMIA**
Origin	Wild	Wild
Region of capture	Andalusia	Andalusia
Year	2000	2000
Number of breeders	44	11
Type of food	Fish / Shellfish	Fish
Conservation of food	Fresh / Frozen	Fresh / Frozen
Meals per week	3	4
Initial weight (g)	339	112
Final weight (g)	9000 – 11000	11700 – 15000
Stock density ($kg.m^{-3}$)	1.7	0.1
SPAWNING INDUCTION		
Variable	**IFAPA**	**LIMIA**
Year of induction	2006	2006
Spawning tanks (m3)	25	10
Temperature (°C)	18.7	18.5
Salinity (g l^{-1})	35	37
Breeders treated (M/F)	1/2	2/1
Mean weight (kg)	11	10
Hormonal treatment (LHRH9	Injections	Injections
Doses of sGnRHa	50 - 25 µg kg^{-1}	10 - 5 µg kg^{-1}

Table 1. Different conditions in maintaining of the broodstock at the two centres.
Taula 1. *Diferents condicions en el manteniment de l'estoc de reproductors als dos centres.*

them with an Olympus stereomicroscope. The real percent fertilization success (RFS) was calculated as the total number of spawned eggs(TE) minus the total number of dead eggs (TDE) multiplied by the total spawned eggs^{-1}(TE) multiplied by 100 [(TE-TDE)xTE^{-1}x100], while the apparent fertili-zation success (AFS) was the number of floating eggs minus the eggs that died during the incubation period (IDE) multiplied by the number of floating eggs^{-1} [(FE-IDExFE^{-1}x100]. Hatching success was estimated as the mean apparent hatching rate (AHR) calculated as the number of hatched larvae (HL) multiplied by the number of floating eggs^{-1} multiplied by 100 (HLxFE^{-1}x100).

Floating eggs were separated and disinfected with a 1% iodoform solution prior to transfer to 400 L conical open circulation tanks for incubation. These tanks were maintained at ambient temperature (20° C) and 37 g L^{-1} salinity. Post-hatching yolk larvae were transferred to 1.2 m^3 tanks containing 1 µm lightly aerated filtered sea water, and the final larval density was 50 larvae L^{-1} (for details of post-hatching and nursery phase conditions see Table 2). Tanks were equipped with surface skimmers and surface drainage to clean the surface oil film and prevent swim bladder development. From day 1 to 7, tanks were equipped with a very low continuous 1 µm filtered sea water overflow (12 L hour^{-1}) that resulted in a 25% day^{-1} water exchange. Then sea water flow was increased progressively until 3 L min^{-1} from day 8 onwards. During larval rearing the temperature ranged between 19 and 23° C. At 35 days post hatch (DPH), fish were harvested from the 1200 L tanks and counted. Surviving fish were then placed in 10 m^3 nursery tanks until day 68. The bottom of the tanks was siphoned daily for cleaning from 1 to 68 DPH, and dead fish were counted. After the nursery phase, 68 day-old fish (2.85 ± 1.23 g mean weight) were stocked in a 12 m^3 sea cage for on-growing. Survival at 68 DPH was estimated by subtracting the daily dead fish from the number of fingerlings counted at 35 DPH.

From 2 to 16 DPH, larvae were fed with rotifers *Brachionus rotundiformis* (Tschugunoff) cultured with yeast and enriched with DHA protein selco (INVE Aquaculture, Belgium) at a density of 10-20 individuals mL^{-1}, plus green water

HATCHERY PHASE		
CENTRE	IFAPA	LIMIA
Interval duration (DPH)	2 - 29	2 - 35
Volume of tanks (m3)	0.7	1.2
Light intensity (lux)	800 – 1000	500-550
Min.- max. temp. (° C)	21 - 25	19 - 23
Salinity (g L-1)	39	37
Density (larvae L-1)	50	50
NURSERY PHASE		
CENTRE	IFAPA	LIMIA
Interval duration (DPH)	29 - 60	35 - 68
Volume of tanks (m3)	10 m	10

Table 2. Meagre different rearing conditions during the experiences at IFAPA and LIMIA.
Taula 2. *Diferents condicions de cultiu durant les experiències al IFAPA i al LIMIA.*

composed of *Nanocloropsis gaditana* and *Isochrisys galvana* at a density of 80000 - 100000 cells mL^{-1}. Rotifer density was estimated twice a day and adjusted accordingly to maintain the desired concentration. From 10 to 29 DPH, larvae were fed *Artemia* nauplii (grade AF480, INVE aquaculture) at 1-2 nauplii mL^{-1} followed by *Artemia* metanauplii (grade EG, INVE aquaculture) enriched with DHA selco (INVE aquaculture). From day 23, meagre fry were fed with a commercial weaning diet (INVE NRD 2/4). The quantity of *Artemia* given to larvae was adjusted daily in accordance with their feeding behaviour.

Every day up to 17 DPH, a sample of 10 larvae was sacrificed with excess MS-222 for microscopic examination, and from then on every two days up to 30 DPH. The total length (TL), yolk sac length and oil globule diameter were measured. Every two days samples were pre-weighed and then dried at 60° C for 24 h. Dry weights were determined after cooling *in-vacuo* for 1 h. Specific growth rates (% day^{-1}) were calculated using the formula $SGR = 100 \times (\ln DW - \ln DW_0) \times t^{-1}$ (Wootom, 1990), where DW and DW_0 are the final and initial dry weights and t the time period in days.

IFAPA (Andalusia)

Maintenance of breeders

On arrival to the research centre fish were weighed, pit tagged and kept in a 250 m^3 tank under a natural thermal and photoperiod regime. Feeding was carried out three times a week with fresh or frozen fish and shellfish to apparent satiation.

In March 2006, fish were anaesthetised in order to determine their sex, weight and length. Males were examined for free milt by applying pressure to the abdomen, and an ovarian biopsy was carried out on females with a 1 mm diameter nasogastric catheter. 5 females with a mean weight of 10.4±2.2 kg and 3 males with a mean weight of 11.2±6.8 kg were separated from the rest of the broodstock and kept in a 25 m^3 tank.

Hormonal induction

In June 2006, 2 females were injected with [D-trip6]-sGnRHa (Sigma Co., St Louis, Missouri) at 50 µg kg^{-1} and 1 male was injected with half that dose. At that time the mean water temperature and salinity in the holding tank were 18.7ºC and 35 g L^{-1} respectively. A 250 L egg collector with a 500 µm net was installed in the tank drain to collect the eggs. Once spawning occurred, the number of viable and non-viable eggs, fertilization success and egg diameters were determined as described above for LIMIA.

Larval rearing

Estimates of the number of eggs were made volumetrically by taking ten 1 mL aliquots of well mixed eggs from 5 L plastic containers. Floating eggs were separated and incubated in 0.3 m^3 conical tanks, which were moderately aerated and had continuously flowing sea water at 21.3º C and 39 g L^{-1} salinity. Egg quality parameters were evaluated using the same calculations described above for LIMIA. Hatching yolk-sac larvae were transferred to 0.7 m^3 tanks containing lightly aerated seawater and equipped with skimmers to remove surface films. Larvae were maintained in a closed circulation system for 9 days at 800-1000 lux and a density of 50 larvae L^{-1}. After this period the tanks received a 25% day^{-1} seawater exchange and were kept under a natural photoperiod. On day 29, fish were placed in a 10 m^3 tank,

and fish were counted on day 60.

Rotifers *Brachionus plicatilis* (Muller) enriched with *Isochrysis galbana* were fed to larvae from 2 to 17 DPH, along with green water (*Nannocloropsis gaditana* and *Isochrysis galbana*). Rotifer densities were estimated daily and maintained at 5-20 individuals mL^{-1}. From 8 to 10 DPH, larvae were fed *Artemia* nauplii at 0.5-1 nauplii mL^{-1} and from 10 to 39 DPH, *Artemia* metanauplii enriched with *Isochrysis galbana*. The quantity of *Artemia* given to larvae was adjusted daily in accordance with the feeding behaviour of the larvae. Dry food was given from 23 DPH and weaning was completed on 39 DPH.

A sample of 10 larvae was examined microscopically each day until 29 DPH. Total length (mm) was measured using a stereo microscope fitted with an ocular micrometer. The dry weight was determined by drying larvae at 60° C for 24 hours. Specific growth rates (% day^{-1}) were calculated using the same formula described above for LIMIA.

Statistical analysis

Data are presented as mean ± SD (standard deviation of the mean). Tukey's test was used to compare differences in SGR between LIMIA and IFAPA. Statistical analyses were performed using SPSS 11.5.

Results

Induced spawning

Histological analysis of 3 year old meagre gonads showed that 50% of the females sacrificed in February 2003 had developed ovaries at the final stage of vitellogenesis (Fig. 1) and 100% of the males had free milt (Fig. 2). The remaining 50% of females had ovaries in the initial stage of vitellogenesis. Therefore, vitellogenesis appeared to progress in the normal time frame; however, the final process of oocyte maturation and ovulation did not occur.

Of the 11 meagre in the broodstock examined at LIMIA, only 1 female had fully vitellogenic oocytes (mean diameter of largest oocytes > 500 µm), while 5 males had running milt that could be detected by applying light pressure to the abdomen. It was not possible to determine the sex of the rest of the broodstock. The vitellogenic female (13.0 kg) and two of the running milt males (12.4 kg and 10.0 kg) were injected and kept together in the reproduction tank separated of the rest of the broodstock. Spawning occurred 38 hours after the first injection (HAI). An estimated 1.207.000 eggs at the blastula stage were collected 6 hours later, with a RFS of 57.4 % (Table 3). A second spawn was collected 48 hours later (444.500 eggs), and a third one 110 HAI (149.000 eggs), although these two last spawns were unviable. The second injection, seven days after the first one, led to the release of 929.840 eggs with a RFS of 76.93 %. As with the first injection, two more spawns were released 62 (180.000 eggs) and 86 HAI (10700 eggs). In this case the spawns were viable but the RFS was not calculated as the eggs were not incubated.the AFR was 74.45%. With the last injection, 14 days after the first one, fish only released 106.000 eggs (Table 3). The relative fecundity (RF) was 232.849.23 eggs kg^{-1} obtained from a single female injected three times in a 15 day period (77616.41 eggs kg^{-1} inj^{-1}). The AFR of the spawns incubated at LIMIA was 71.1%, and larval AHR was 86.4%. Out of the 43 specimens of meagre examined at IFAPA, 5 were vitellogenic females (9.4±1.9 kg) with vitellogenic oocytes (mean diameter of the largest

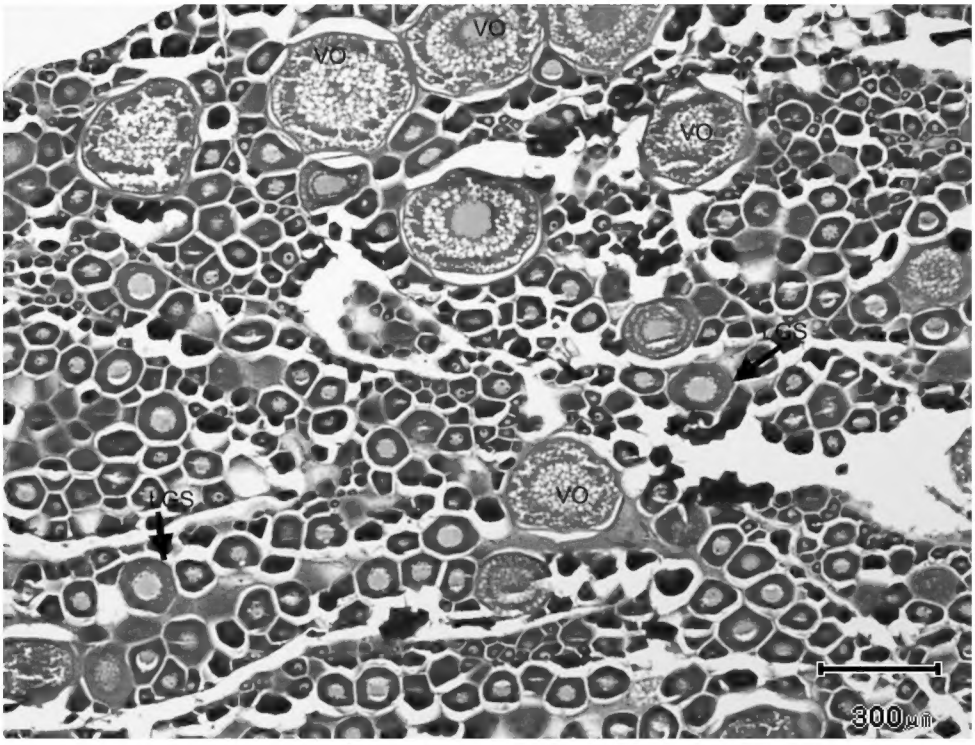

Fig. 1. Transverse section of *A. regius* ovary at final stage of vitelogenesis. Note the presence of some vitellogenic oocytes (VO) and cortical alveolus oocytes (CA) in the ovaric lamella interspersed between a large population of remaining oocytes at the primary growth stage.
Fig. 1. *Secció transversal de l'ovari d'* A. regius *a l' estat final de la vitelogènesi. S'observa la presència d'algun ovòcit vitelogènic (VO) i ovòcits alvèol corticals (CA) a la lamel·la ovàrica dispersos entre una gran quantitat d'ovòcits que romanen a l'estadi primari de creixement.*

Centre	Hormonal induction	Spawning (HAI)	Total eggs	% F	% H
LIMIA	First	38	1207000	65.7	57.4
	First	86	445000	0	0
	First	110	149000	0	0
LIMIA	Second	38	929840	84.3	76.9
	Second	62	180000	75.4	NV
	Second	86	107000	73.5	NV
LIMIA	Third	38	106000	77.7	NV
IFAPA	First	38	288200	0	0
	First	62	459360	93.2	47.7

Table 3. Meagre spawning results at LIMIA and IFAPA in 2006. HAI: hours after injection. RFS: real fertilization success (%). AFS: apparent fertilization success (%). NV: not valued
Taula 3. *Resultats de la posta al LIMIA i al IFAPA al 2006. HAI : hores després de la injecció. RFS: Taxa real de fertilització (%) AFS: Taxa aparent de fertilització. NV; no valorat.*

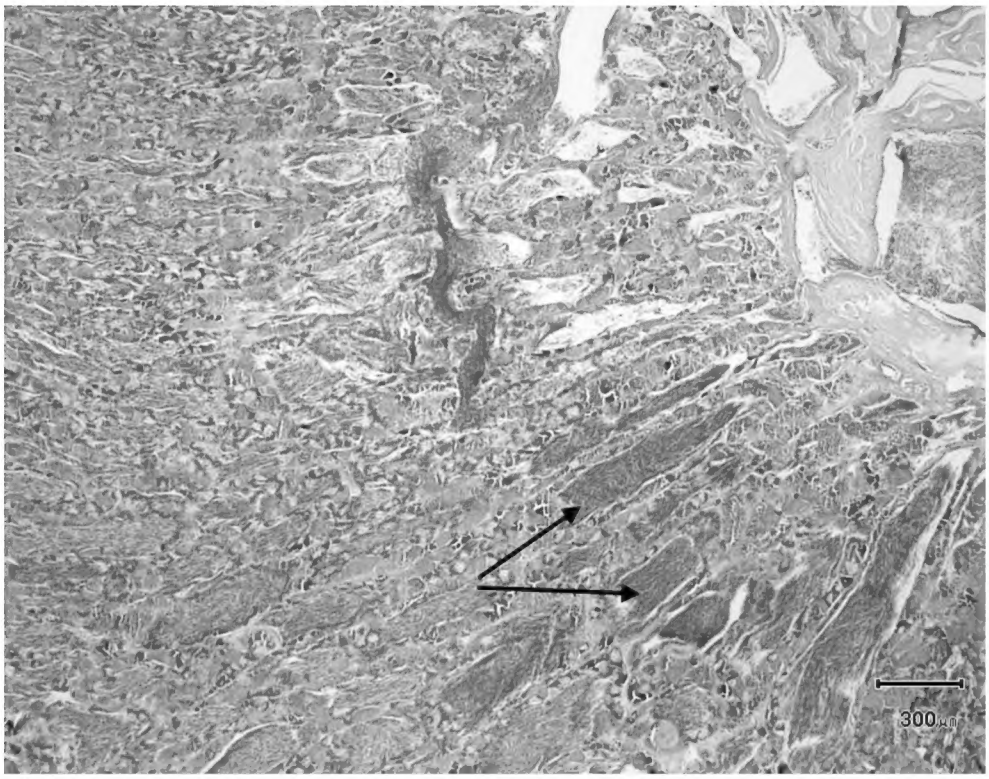

Fig. 2. Transverse section of *A. regius* spawning testis. Note the presence of abundant espermatozoa. (arrow) in the spermatogenic tubules

Fig. 2. *Secció transversal d'un testicle en posta de* A. regius. *S'observa la presència d'abundants espermatozous (fletxa) dins els túbuls espermàtics.*

oocytes > 500 µm), 25 were running ripe males (9.7±1.4 kg) and the remaining 13 were undetermined. Of these examined fish, 2 females (11.1±1.6 kg) and 1 male (11.6 kg) were each given a single injection and kept together in the reproduction tank. The spawn starts 38 hours after induction. After 44 hours a total of 288.200 unviable eggs were collected, followed by 459360 eggs a further 24 hours later, of which 235.192 were floating eggs. This led to a RFS of 47.7 % (Table 3). The relative fecundity (RF) was 20691.9 eggs kg^{-1} of female with only one injection. The AFS obtained at IFAPA was 83.3 %, and larval AHR was 91.1 %.

Embryonic development

Fertilized eggs were translucent and buoyant with a mean diameter of 904±49 µm at LIMIA and 850±20 µm at IFAPA. They had multiple non-pigmented oil globules which coalesced into a single globule at the C-shaped embryo stage (Fig.3).

Eggs were collected early in the morning in the blastula stage, incubated at 20.1±0.4ºC and hatched 27 hours later.

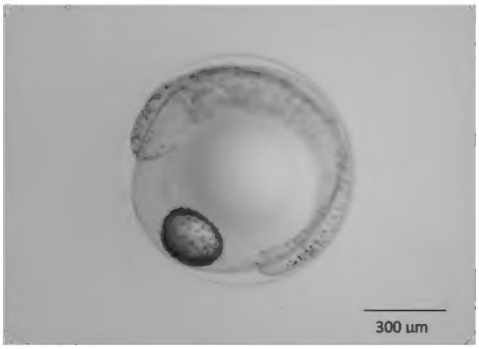

Fig. 3. Egg in embryonic phase.
Fig. 3. *Ous en fase embrionària.*

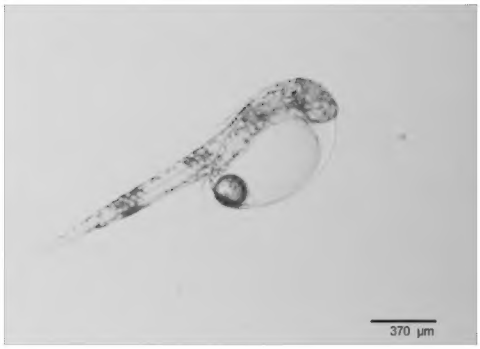

Fig. 4. Recently hatched larvae
Fig. 4. *Larva recent eclosionada.*

Larval development

At LIMIA, the length of the newly hatched larvae was 2.22±0.022 mm and they had a mean dry weight of 63±1 µg. The larvae were buoyant, transparent with many chromatophores, and contained a single pigmented oil globule (219.5± 0.00µm) at the caudal end of the yolk sac (1.137±0.057 mm) (Fig.4). The body was surrounded by the primordial fin and the gut which was looped on the ventral side and had no communication with the exterior. After 24 hours the otoliths were clearly visible, the larvae had already consumed half of the yolk sac, and the eyes were easily differentiated. By the second DPH the yolk sac was almost completely reabsorbed, the gut had an opening to the exterior via the anus, and the mouth had started to open, though it still lacked movement. At 3 DPH larvae were 3.3±0.09 mm long, the mouth was mobile and rotifers were observed in the digestive track of some individuals. Pectoral fins started to grow on 4 DPH. On 5 DPH the first larvae with functional swim bladders were observed, and by 13 DPH swim bladders were functional in 91% of larvae. Metamorphosis began by 25 DPH when larvae were approximately 14 mm long, and by 30 DPH 90% of larvae had metamorphosed and were swimming on the bottom of the tank. This transformation marked the end of the larval phase. On 35 DPH fish were harvested from the 1.2 m^3 litre tanks and counted, with survival estimated at 11.75%. Individuals were stocked in a 10000 litre fibreglass nursery tank until they reached 2.85±1.23 g mean weight at 68 DPH, after which they were transferred to sea cages for on-growing. On 60 DPH the estimated fish survival was 6.58 %.

At IFAPA, hatched larvae had a mean total length of 2.82±0.37 mm and a mean dry weight of 53±6 µg. At 3 DPH larvae were 2.93±0.13 mm long. When fish reached 29 DPH, they were placed in a 10 m^3 tank. On day 60, fish survival was 16 %.

At both locations growth was very fast, and post-larvae reached a mean length of 15.11±3.49 mm and a dry weight of 9571.4±6389.5 µg in 30 days at LIMIA and 11.66±0.96 mm and 5051.0±1526.2 µg in 29 days at IFAPA.

The following equations represent growth in larval length and dry weight (also see Fig. 5):

LIMIA: $TL = 2.405.e^{0.058.DPH}$ (R^2= 0.943); $DW = 6.394.\ e^{0.244.DPH}$ (R^2= 0.983)

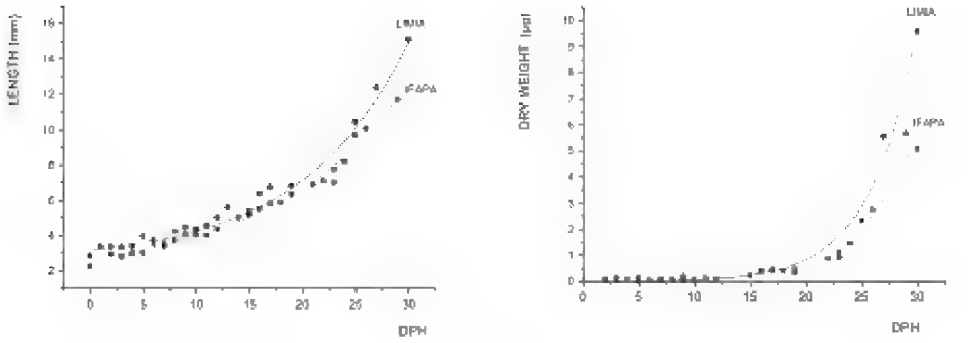

Fig. 5. Growth in larval length (left) and dry weight (right) in LIMIA and IFAPA.
Fig. 5. *Creixement larvari en longitud(esquerra) i pes sec (dreta) al LIMIA i IFAPA.*

IFAPA: TL= $2.323.e^{0.054.DPH}$ (R^2= 0.943); DW= $11.785.e^{0.206.DPH}$ (R^2= 0.965)

Where TL is the total larval length and DW is the dry weight. The mean SGR during larval development was 18.29±6.16% day^{-1} at LIMIA and 17.37±7.22% day^{-1} at IFAPA. There was no difference in SGR between LIMIA and IFAPA (P>0.05).

Discussion

The present study provides data on induced spawning and larval rearing of meagre in captivity in two research centres in Spain under different conditions. In the Gironde estuary wild meagre adults aggregate from May to July (Quero, 1989), and the spawning period generally takes place throughout June and the beginning of July (Quero and Vayne, 1987) when the water temperature in the estuary changes from 16° C to 21° C meagre is therefore a typical spring spawning species that spawns when both the photoperiod (12-14.5 h $light.day^{-1}$) and temperature (16°-21°C) are increasing. In captivity, first developing oocytes were observed in 3 year old females, while males matured one year earlier (pers. obs.). This is a common pattern in the reproductive biology of gonochoristic species in which size at first maturity differs between sexes, with males reaching maturity at a smaller size than females (Gonzalez-Quirós *et al.,* 2011). The same characteristics have been observed in other sciaenids such as *A. japonicus* (Silberschneider *et al.,* 2009), brown meagre *Sciaena umbra* (L.) (Grau *et al.,* 2009) *U. cirrosa* (Mylonas *et al.*, 2000), Madagascar meagre *Argyrosomus hololepidotus* (Lacepède) (Battaglene and Talbot 1994) and orangemouth weakfish *Cynoscion xanthulus* (Jordan and Gilbert) (Prentice and Colura, 1984). Spontaneous spawning in meagre *was* not observed in either the LIMIA or IFAPA laboratories. This species therefore does not appear to reach final oocyte maturation in captivity, which is the most common reproductive dysfunction in cultured marine species. Fish exhibiting this type of dysfunction undergo normal vitellogenesis, but with the onset of the spawning season the developing oocytes fail to initiate ovulation and instead undergo atresia (Zohar and Mylonas, 2001).

Different reproductive hormones have been used since the beginning of commercial aquaculture to stimulate reproductive processes and control the spawning of broodstock, including injections of gonadotropic hormones (GTH), pituitary extracts containing GTH, human chorionic GTH and gonadotropin-releasing hormones (GnRH). In some sciaenid species which are unable to breed spontaneously in captivity, treatment with hormones has proven to be an effective method for inducing spawning, such as *S. ocellatus* treated with luteinizing hormone releasing hormone (LHRH) (Thomas and Boyd, 1988; Gardes *et al.,* 2000), *A. hololepidotus* (Battaglene and Talbot, 1994) and *Micropogonias furnieri* (Desmarest) (García-Alonso and Vizziano, 2004) injected with human corionic hCG, and *U. cirrosa* injected with GnRHa (Barbaro *et al.,* 2002). Currently, most commercial fish farmers choose GnRH analogues due to their many advantages. These GnRH peptides are small and use the endocrine pathways of the fish similarly to the native GnRH peptide because they travel from the injection to the pituitary through the blood, and bind to the pituitary receptors with a greater affinity than native peptides (Powell *et al.,* 1998).

Meagre is tolerant of captivity and completes maturation to an advanced stage; it is therefore possible to apply induced spawning protocols successfully (Duncan *et al.*, 2008). The authors are not aware of any existing data concerning the fecundity of meagre in the wild. In our study *meagre* was induced with injections of two different sGnRHa, which led to spontaneous spawning in tanks 38 hours after induction, with eggs released naturally by females for fertilization by males. These results were similar in both centres. Duncan *et al.* (2008) did not report data on the time that elapsed from the injection to spawning in his experiences, but comparable results were obtained for the sciaenids, *A. hololepidotus,* 32 hours at 25ºC and 34 hours at 22ºC (Bataglenene and Talbot, 1994), and *U. cirrosa*, 34 hours at 24ºC (Francescon and Barbaro, 1999). Hand stripping was not necessary at either of the two centres, which is of great value for aquaculture husbandry as stripping is very time-consuming, laborious and stressful for the fish. Fertilization success and egg viability are also highly variable for batches that are stripped from fish (Norberg *et al.,* 1991; Bromage *et al.,* 1994). Francescon and Barbaro (1999) and Duncan *et al.* (2008) obtained spawning without stripping; however, manual stripping was necessary to obtain eggs and sperm in the induction of *A. hololepidotus* (Battaglene and Talbot, 1994). As we have seen at LIMIA in this study, a mature meagre female can release more than 90000 eggs kg^{-1} in a single spawning event. This high fecundity has also been observed in *A. hololepidotus* (Aqua KE Government Documents, 2004) and *U. cirrosa* (Francescon and Barbaro, 1999) and by Duncan *et al.* (2008) also for meagre. However, at IFAPA the fecundity was lower. The hormonal induction protocol at this centre was carried out one month later than at LIMIA, and the low values obtained were probably due to the fish being injected late in relation to the natural spawning season of the species. Photoperiod and temperature are the triggers of gonadal maturation processes in fish. Francescon and Barbaro (1999) working with *U. cirrosa* under experimental conditions described considerable differences in fecundity depending on temperature and photoperiod. The maxim value (GSI> 4) of the gonadosomatic index (GSI) of the meagre female reared at LIMIA occurred

during May while in June this index fell to a value below 2.5. The GSI provides a useful general indication of seasonal trends (Wilk *et al.*, 1990), but it needs to be corroborated using histological criteria (Matsuyama *et al.*, 1987, West 1990). Histological studies show that in May, 100% of ovaries have a large number of vitellogenic oocytes capable of being hydrated and released. However, in June only 20% of the ovaries are found in this stage, the other 80% have more than half of the vitellogenic oocytes in the degenerative state (atresia) and they can no longer be released (Gil unpublished data). Therefore, the number of vitellogenic oocytes that these females can release after a hormonal induction will be reduced to less than half compared with the females injected in the previous month. This results could also be related to the possible effect of a dopamine antagonist that was co-injected at LIMIA but not at IFAPA; however, the experiences carried out by Duncan *et al.* (2008) in inducing spawning in meagre with GnRHa without dopamine antagonist clearly show that, as in most commercially important cultured marine fish, there is no dopaminergic inhibitor in this specie.

The average diameter of eggs was 904±49 µm at 37 g L^{-1} at LIMIA, and 850±20 µm at 35 g L^{-1} at IFAPA, which is consistent with other observations made for meagre (Gamsiz and Neke, 2008) and *U. cirrosa* (Zaiss *et al.*, 2006). Development time is a function of incubation temperature, while egg diameter is correlated with spawning salinity. Many euryhaline sciaenids spawn in estuarine and coastal waters where their eggs are exposed to a wide range of salinities. For example, spotted weakfish *Cynoscion nebulosus* (Cuvier) produce eggs with a small diameter (600 µm) at 45 g L^{-1} and eggs with a large diameter (860 µm) at 21 g L^{-1} salinity. A similar relationship was observed for laboratory spawned *S. ocellatus,* which produced eggs with a diameter of 1001 µm at 24 g L^{-1}, 950 µm at 28 g L^{-1} and 920 µm at 37 gL^{-1} (Thomas *et al.*, 1995).

The length of newly hatched larvae was similar to observations made by Battaglene and Talbot (1994) for *A. hololepidotus*. Mouth opening was completed at 3DPH, which is slightly earlier than expected for other commonly cultured species, such as gilthead seabream *Sparus aurata* L. and common dentex *Dentex dentex* (L.) in which it occurs at 4 DPH (Elbal *et al.,* 2004 and Santamaría *et al.,* 2004 respectively), and turbot *Psetta maxima* (L.) in which it occurs at 4-5 DPH (Segner *et al.* 1994). Our results do, however, agree with findings made by Gamsiz and Neke (2008) for meagre and Zaiss *et al.* (2006) for *U. cirrosa*. Presumably, this apparently early mouth opening was due to the faster growth of *meagre* at the higher rearing temperature. Complete yolk absorption also took place simultaneously with mouth opening.

In terms of the larval rearing stages, meagre appears to fulfil many of the prerequisites for an aquaculture species: the larvae have a large mouth, a very fast growth rate, high survival rates and are easy to handle. The same characteristics have also been described for *U. cirrosa* (Mylonas *et al.,* 2000) and the growth rate was similar to that found for *S. occellatus* (Holt 1981). At 30 DPH the mean size of the post-larva was 15.11±3.49 mm, which is higher than those described by Lozano *et al.* (2004) for redbanded *Pagrus auriga* (Valenciennes) and by Zaki *et al.* (2009) for *Sparus aurata* (L.).

In the present study, the ontogenic development of meagre larvae was very similar to that of *A. hololepidotus* and *S.*

ocellatus (Holt *et al.,* 1981; Battaglene and Talbot, 1994). This similarity will be helpful for establishing hatchery techniques for meagre because techniques that have already been developed for other sciaenids can be adapted. The feeding schedules used in this study were comparable to those used for *A. hololepidotus* (Battaglene and Talbot, 1994), and larvae reacted very positively to dry food given from day 23, which indicates that they could be weaned earlier.

Early larval mortality was not quantified in this study, but cannibalism was observed in both centres from day 15 onwards. Cannibalism is a problem common to most intensively cultured sciaenids (Arnold *et al.,* 1976; Soletchnik *et al.,* 1988; Orhun, 1989; Battaglene and Talbot, 1994) and may be associated with problems of starvation, size dispersion, population density and illumination (Dou, 2000; Herrera *et al.,* 2008). In our case, size differences and larval density may have been the main cause of this behaviour.

In conclusion, *Argyrosomus regius* is a very important candidate for aquaculture diversification. The adults of this species have calm behaviour and are therefore easy to handle. They adapt to the conditions of sea cages without problems. Moreover, their reproductive process can be controlled by hormonal induction with sGnRHa, larvae are relatively easy to rear and juveniles grow fast. For all these reasons we believe that meagre is a species that is well suited to aquaculture and that large quantities of juveniles could be produced easily.

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